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(54) **Coated pesticidal agents, processes for their preparation and compositions containing them**

(57) The present invention provides a coated pesti-
cidal agent and processes for its preparation. The
present invention also provides a wettable powder pes-

ticidal composition containing the coated pesticidal
agent.

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Description

Certain pesticidal agents are inactivated by ultraviolet radiation from the sun. Because those pesticidal agents are useful for the control of undesirable pests and are applied in areas where they will be exposed to ultraviolet radiation, there is a need for photostable compositions containing those agents.

To prevent ultraviolet inactivation of pesticidal agents, compositions have been prepared which contain ultraviolet absorbers and/or reflectors and a pesticidal agent.

U.S. Patent No. 3,541,203 describes a protected virus composition for insect control. The preferred composition includes a virus, an actinic light absorbing material and a polymeric binder material. The patent discloses that to bind the admixture of an actinic light absorbing material and a virus together with an ethyl-cellulose polymeric material, the admixture is combined with an ethylcellulose in toluene solution. The resultant mixture is agitated, treated with polybutadiene and poured into petroleum distillate which causes the ethylcellulose to solidify to yield very small particles of ethylcellulose polymeric material having substantially homogeneously enclosed within the particles the admixture of the actinic light absorbing material and the virus. The particles are then washed several times with additional petroleum distillate to completely remove residual amounts of the liquid polybutadiene material. Unfortunately, the process used to prepare the preferred compositions of U.S. Patent No. 3,541,203 is not entirely satisfactory because it requires the use of toxic materials and numerous washing steps with flammable solvents.

U.S. Patent No. 4,948,586 discloses a microencapsulated insecticidal pathogen. Four microencapsulated compositions are shown to decrease the photoinactivation of *Autographa californica* NPV. However, the microencapsulated compositions only retained from 30.7 to 71.43% of the original activity before being exposed to sunlight. U.S. Patent No. 4,948,586 discloses a method of preparing microencapsulated insecticidal pathogens which has numerous steps and is both time consuming and laborious. It is apparent that neither the process nor the microencapsulated insecticidal pathogens described in U.S. Patent No. 4,948,586 are entirely satisfactory for protecting insecticidal pathogens from the effects of ultraviolet radiation.

The present invention seeks to provide a coated pesticidal agent which retains a significant amount of its original activity after exposure to ultraviolet radiation.

The present invention also seeks to provide simple, less arduous processes for the preparation of coated pesticidal agents which are more suitable for commercial manufacture.

The present invention further seeks to provide a wettable powder pesticidal composition containing a coated pesticidal agent.

SUMMARY OF THE INVENTION

The present invention describes coated pesticidal agents which retain a significant amount of their original activity after exposure to ultraviolet radiation.

The coated pesticidal agents of the present invention comprise a pesticidal agent core surrounded by a matrix which comprises about 2 to 25% by weight of a pH-dependent polymer, 0% to about 5% by weight of a plasticizer, about 5 to 45% by weight of an ultraviolet protector, 0% to about 75% by weight of a stilbene compound, 0% to about 10% by weight of a disintegrating agent, and 0% to about 10% by weight of a glidant.

The present invention further provides processes for the preparation of coated pesticidal agents, and wettable powder pesticidal compositions comprising the coated pesticidal agents.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts a schematic representation of the AcMNPV genome showing the location of the *egt* gene. In Figure 1A the entire AcMNPV genome is presented in map units and as Eco RI and Hind III restriction maps. Figure 1B depicts a more detailed map of the region located between map units 7.6 and 11.1 and shows the location of the *egt* gene.

Figure 2A depicts a schematic representation of the *egt* gene region, which shows key restriction sites between map units 8.3 and 9.8 in the AcMNPV genome. Figure 2B depicts the organization of open reading frames in the three forward (1, 2, 3) and three reverse (1', 2', 3') reading frames of the AcMNPV genome between map units 8.3 and 9.8. The large open reading frame in frame 2 marks the position of the protein coding region of the *egt* gene.

Figure 3 depicts a schematic view of the organization and derivation of the DNA fragments used to assemble the (unloaded) AcMNPV V8 transfer vector NF4. Figure 3A depicts the manner in which fragments A - D are joined to form NF4. Figure 3B depicts a schematic representation of the process used for the preparation of Fragments C and D. The arrows above the linear restriction map of the AcMNPV V8 Eco RI "I" fragment depict the location and transcriptional polarity of the major open reading frames (ORFs) located between map units 0.32 and 5.83 in the AcMNPV genome. The symbols "H" and "E" depict the positions of the recognition sites for restriction endonucleases Hind III and Eco RI, respectively.

Figure 4 depicts detail of the construction of the plasmid pBS ADK-AaIT, which contains the heterologous adipokinetic hormone gene signal sequence and a codon optimized cDNA sequence encoding AaIT.

Figure 5 depicts a portion of a modular expression vector with Bsu 36I and Sse 8387I sites at opposite ends of an expression cassette containing a promoter module, a polylinker module and a 3' UTR module. The polylinker module contains an Esp 3I recognition site. The region bounded by the outermost Bsu 36I and Sse 8387I sites is defined as the virus insertion module.

Figure 6 depicts the polymerase chain reaction (PCR) strategy for the amplification of a adipokinetic hormone gene signal/codon optimized AaIT gene, which is then digested with Bam HI.

Figure 7 depicts a schematic representation of a modular expression vector (AC0075.1) formed by inserting the adipokinetic hormone gene signal/codon optimized AaIT into pMEV1.1, which contains the AcMNPV DA26 promoter.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides processes for the preparation of coated pesticidal agents which avoid the problems associated with the methods known in the art.

One process of the present invention comprises:

a) blending a mixture of a pH-dependent polymer, a pesticidal agent, optionally a plasticizer, an ultraviolet protector, optionally a stilbene compound, optionally a disintegrating agent and optionally a glidant in an organic solvent such as acetone, C₁-C₃ alcohol, or combination thereof to produce a homogeneous suspension;

b) drying the homogeneous suspension of step (a); and optionally

c) milling the dried material of step (b).

Advantageously, the foregoing process of the present invention provides an efficient two or three step process for the preparation of coated pesticidal agents which avoids the use of multiple mixing and emulsifying steps, and avoids the use of numerous washing steps with flammable solvents. Further, the above process of this invention preferably utilizes an acetone/C₁-C₃alcohol solution which does not significantly inactivate pesticidal agents such as insecticidal pathogens. Other organic solvents taught in the art, such as methylene chloride, may significantly inactivate pesticidal agents such as insecticidal pathogens.

While the foregoing process is believed to be a significant advance over prior processes, a preferred process which avoids the use of the organic solvents has also been invented.

Uniquely, it has been found that coated pesticidal agents may be prepared by an aqueous process which comprises:

a) preparing an aqueous mixture of a pH-dependent polymer and optionally a plasticizer;

b) dissolving the pH-dependent polymer by adjusting the pH of the mixture of step (a) to about 8.5 to 10 with a base;

c) adding a pesticidal agent, an ultraviolet protector, optionally a stilbene compound, optionally a disintegrating agent and optionally a glidant to the solution of step (b), and blending to produce a homogeneous suspension;

d) drying the homogeneous suspension of step (c); and optionally

e) milling the dried material of step (d).

Advantageously, the aqueous process of this invention does not require the use of multiple mixing and emulsifying steps, and does not require the use of numerous washing steps with flammable solvents and further does not require the use of organic solvents.

The coated pesticidal agents of the present invention preferably have a particle size less than about 20µm and more preferably have a particle size of about 2µm to 10µm. The homogeneous suspensions of this invention may be dried using any conventional drying technique. Preferably the suspensions are spray dried or air dried.

Preferred coated pesticidal agents prepared by the processes described hereinabove are those comprising a pesticidal agent core surrounded by a matrix which comprises about 2 to 25% by weight of a pH-dependent polymer, up to about 5% by weight of a plasticizer, about 5 to 45% by weight of an ultraviolet protector, up to about 75% by weight of a stilbene compound, up to about 10% by weight of a disintegrating agent, and up to about 10% by weight of a glidant.

The ratio of the pesticidal agent to the matrix is preferably about 1:1 to 1:10. And the ratio of acetone to the C₁-C₃alcohol is preferably about 1:9 to 9:1 and more preferably about 1:4 to 2:3. C₁-C₃alcohols suitable for use in the

organic process include methanol, ethanol, isopropanol and n-propanol with isopropanol being preferred.

Pesticidal agents suitable for use in the present invention include chemical and biological insecticides, acaricides, nematocides and fungicides or mixtures thereof which are inactivated by ultraviolet radiation. Preferred pesticidal agents are insecticidal pathogens such as viral pathogens, bacterial pathogens and fungal pathogens. Viral pathogens suitable for use include wild gypsy moth NPV; *Autographa californica* NPV's such as AcMNPV E2, AcMNPV L1, AcMNPV V8, V8vEGTDEL and V8vEGTDEL-AalT; Douglas fir tussock moth NPV; European pine saw fly NPV; and *Heliothis zea* NPV. Most preferred pesticidal agents for use in this invention are wild gypsy moth NPV, AcMNPV E2, AcMNPV L1, AcMNPV V8, V8vEGTDEL, V8vEGTDEL-AalT, and *Heliothis zea* NPV.

AcMNPV E2 is described in EP 621337 published October 26, 1994.

pH-Dependent polymers suitable for use in the present invention include polymers which are essentially insoluble below about pH 5 such as methacrylic acid and methyl methacrylate copolymers, maleic anhydride and styrene copolymers and the like or mixtures thereof. Preferred pH-dependent polymers include methacrylic acid and methyl methacrylate copolymers, methacrylic acid and methyl methacrylate copolymer mixtures and maleic anhydride and styrene copolymers. Most preferred pH-dependent polymers are Eudragit®S (methacrylic acid and methyl methacrylate copolymer wherein the ratio of free carboxyl groups to esters is about 1:2; Röhm Pharma GmbH, Weiterstadt, Germany), Eudragit®L (methacrylic acid and methyl methacrylate copolymer wherein the ratio of free carboxyl groups to esters is about 1:1; Röhm Pharma GmbH) and mixtures thereof.

Plasticizers suitable for use in the present invention include any of the conventional agents known in the art such as polyethylene glycols, polypropylene glycols, diethyl phthalate, dibutyl phthalate, citric acid esters, castor oil, triacetin and the like or mixtures thereof with polyethylene glycols having a molecular weight of about 300 to 1,000 being preferred.

Ultraviolet protectors are used in the present invention to reduce the photoinactivation of the pesticidal agent. Ultraviolet protectors suitable for use include ultraviolet absorbers and ultraviolet reflectors or mixtures thereof. Ultraviolet absorbers include various forms of carbon such as carbon black; benzophenones such as 2-hydroxy-4-methoxybenzophenone (CYASORB®UV 9, Cytec Ind.), 2,2'-dihydroxy-4-methoxybenzophenone (CYASORB®UV 24, Cytec Ind.), 2-hydroxy-4-acryloyloxyethoxybenzophenone (CYASORB®UV 2095, Cytec Ind.), 2-hydroxy-4-n-octoxybenzophenone (CYASORB®UV 531, Cytec Ind.) and the like; and dyes such as congo red, malachite green, malachite green hydrochloride, methyl orange, methyl green, brilliant green, acridine yellow, FDC green, FDC yellow, FDC red and the like. Ultraviolet reflectors include titanium dioxide and the like. Preferred ultraviolet protectors include carbon black, benzophenones, dyes and titanium dioxide with carbon black, CYASORB®UV 9 and CYASORB®UV 24 being most preferred.

Bases suitable for use in the aqueous process of this invention include ammonium hydroxide, alkali metal hydroxides, alkaline earth metal hydroxides and the like with ammonium hydroxide being preferred. Certain insecticidal viral pathogens may be deactivated at a pH greater than 10. Therefore it is preferred to select an amount of a base which will adjust the pH to about 8.5 to 10 to ensure ready solubilization and lessen the chance of deactivation.

Stilbene compounds are used in this invention to enhance pesticidal activity of the pesticidal agent. Stilbene compounds suitable for use in this invention are described in U.S. Patent No. 5,246,936.

Preferred stilbene compounds are the analogues of 4,4'-diamino-2,2'-stilbene disulfonic acid, namely, a Calcofluor White (available from Sigma Chemical Co., St. Louis, Mo.) such as Calcofluor White M2R, Calcofluor White ABT, Calcofluor White LD, Calcofluor White RWP, etc.; a Blancophor (available from Mobay Chemicals, Pittsburgh, Pa.) such as Blancophor BBH, Blancophor MBBH, Blancophor BHC, etc.; an INTRAWITE® (a heterocyclic stilbene derivative, available from Crompton and Knowles Corp., Charlotte, N.C.) such as INTRAWITE®CF, etc.; a Leucophor (available from Sandoz Chemicals Corp., Charlotte, N.C.) such as Leucophor BS, Leucophor BSB, Leucophor EKB, Leucophor PAB, etc.; a Phorwite (available from Mobay Chemicals, Pittsburgh, Pa.) such as Phorwite AR, Phorwite BBU, Phorwite BKL, Phorwite CL, Phorwite RKK, etc. and the like. Blancophor BBH, Calcofluor White M2R and Phorwite AR are the most preferred stilbene compounds.

Disintegrating agents are used in the present invention to shorten the milling time and enhance the particle size reduction of the dried material. Disintegrating agents suitable for use in this invention include salts of the condensation products of formaldehyde with the sulfonation products of polycyclic aromatic compounds, hydrophilic starches such as dextran, carboxy methylcellulose, polyvinyl pyrrolidone and the like or mixtures thereof. Preferred disintegrating agents are salts of the condensation products of formaldehyde with the sulfonation products of polycyclic aromatic compounds such as salts of the condensation products of formaldehyde with naphthalene sulfonates, petroleum sulfonates and lignin sulfonates with the sodium sulfonate of naphthalene formaldehyde condensates such as MORWET®D425 (Witco, Houston, Texas), LOMAR®PW (Henkel, Cincinnati, Ohio) and DARVAN®1 (R.T. Vanderbilt Co., Norwalk, Connecticut) being most preferred.

Glidants are used in the processes of this invention to keep the dried, coated pesticidal agents from sticking together. Glidants suitable for use in this invention include talc, magnesium stearate, calcium stearate, calcium sulfate and the like or mixtures thereof with talc being preferred.

Other additives such as preservatives, stabilizers (trehalose), anti-mold agents, anti-fungal agents, anti-bacterial agents and the like may also be included in the matrix of the present invention. Clearly, anti-fungal agents and anti-bac-

terial agents generally would not be used when fungal pathogens and bacterial pathogens, respectively, are coated.

Preferred coated pesticidal agents of the present invention are those comprising a pesticidal agent core surrounded by a matrix which comprises about 2 to 20% by weight of a pH-dependent polymer, up to about 3% by weight of a plasticizer, about 5 to 35% by weight of an ultraviolet protector, about 25 to 75% by weight of a stilbene compound, up to about 10% by weight of a disintegrating agent, and up to about 10% by weight of a glidant.

The present invention also provides a wettable powder pesticidal composition which comprises about 2 to 25% by weight of a wetting agent; about 2 to 40% by weight of a dispersing agent; about 10 to 70% by weight of a bulking agent; about 1 to 10% by weight of a flow enhancing agent; up to about 20% by weight of a pH-modifying agent; and about 5 to 75% by weight of a coated pesticidal agent which comprises a pesticidal agent core surrounded by a matrix which comprises about 2 to 25% by weight of a pH-dependent polymer, up to about 5% by weight of a plasticizer, about 5 to 45% by weight of an ultraviolet protector, up to about 75% by weight of a stilbene compound, up to about 10% by weight of a disintegrating agent, and up to about 10% by weight of a glidant.

Preferred wettable powder pesticidal compositions of the present invention are those comprising about 2 to 15% by weight of a wetting agent; about 2 to 15% by weight of a dispersing agent; about 10 to 60% by weight of a bulking agent; about 1 to 5% by weight of a flow enhancing agent; up to about 20% by weight of a pH-modifying agent; and about 5 to 75% by weight of a coated pesticidal agent which comprises a pesticidal agent core surrounded by a matrix which comprises about 2 to 25% by weight of a pH-dependent polymer, up to about 5% by weight of a plasticizer, about 5 to 45% by weight of an ultraviolet protector, up to about 75% by weight of a stilbene compound, up to about 10% by weight of a disintegrating agent, and up to about 10% by weight of a glidant.

Wetting agents suitable for use in the present invention include any of the conventional agents known in the art. Preferred wetting agents include anionic agents such as sodium N-methyl-N-oleoyltaurate, octylphenoxy polyethoxy ethanol, nonylphenoxy polyethoxy ethanol, sodium dioctyl sulfosuccinate, sodium dodecyl benzene sulfonate, sodium lauryl sulfate, sodium alkyl naphthalene sulfonate, sodium sulfonated alkyl carboxylate and the like or mixtures thereof. A mixture of sodium alkyl naphthalene sulfonate and sodium sulfonated alkyl carboxylate (MORWET®EFW, Witco) is the most preferred wetting agent.

Dispersing agents useful in the wettable powder pesticidal compositions of this invention include any of the conventional agents known in the art. Preferred dispersing agents are anionic agents such as salts of the condensation products of formaldehyde with the sulfonation products of polycyclic aromatic compounds, sodium lignosulfonate and the like or mixtures thereof with the sodium sulfonate of naphthalene formaldehyde condensates such as MORWET®D425 (Witco), LOMAR®PW (Henkel) and DARVAN®1 (R.T. Vanderbilt Co.) being most preferred.

Bulking agents suitable for use in the compositions of the present invention include natural and synthetic clays and silicates, for example: natural silicas such as diatomaceous earths; magnesium silicates such as talcs; magnesium aluminum silicates such as attapulgites and vermiculites; aluminum silicates such as kaolin, montmorillonites and micas; and hydrated aluminum silicates such as kaolin clay. Preferred bulking agents are hydrated aluminum silicates, aluminum silicates, magnesium silicates and magnesium aluminum silicates with kaolin clay being the most preferred bulking agent. Flow enhancing agents useful in the wettable powder pesticidal compositions of this invention are conventional flow enhancing agents known in the art with silicates such as calcium silicates being preferred.

pH-Modifying agents are used to maintain the pH of aqueous tank-mixes prepared from the compositions of this invention below about pH 5. pH-Modifying agents suitable for use include potassium hydrogen phthalate and organic acids with citric acid being preferred.

The wettable powder pesticidal compositions of the present invention may be prepared by blending a mixture of a wetting agent, a dispersing agent, a bulking agent, a flow enhancing agent and optionally a pH-modifying agent to form a premix. The premix is then blended with a coated pesticidal agent to form the desired wettable powder pesticidal composition of the present invention.

For the control of pests, the wettable powder pesticidal compositions of this invention are diluted with water to form an aqueous tank-mix and the tank-mix is applied directly to the pests, their breeding grounds, food supply or habitat.

Other ingredients such as attractants, stickers, anti-foaming agents and the like may be added to the wettable powder compositions of this invention. However, those additional ingredients are generally added separately to the tank-mix. An adjuvant or mixture of adjuvants may also be added to the tank-mix.

In order to facilitate a further understanding of the invention, the following examples are presented primarily for the purpose of illustrating more specific details thereof. The invention should not be deemed limited thereby except as defined in the claims.

Unless otherwise noted, standard molecular biological techniques are utilized according to the protocols described in Sambrook et al. Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Press, Cold Spring Harbor, N. Y. (1989). Standard techniques for baculovirus growth and production are utilized according to the protocols described in Summers and Smith A Manual Of Methods For Baculovirus Vectors and Insect Cell Culture Procedures, Dept. of Entomology, Texas Agricultural Experimental Station and Texas A & M University, College Station, Texas 77843-2475, Texas Agricultural Experiment Station Bulletin No. 1555 (1987).

A deposit of AcMNPV V8 has been made under the Budapest Treaty and given ATCC No VR-2465.

EXAMPLE 1

Preparation of coated V8vEGTDEL polyhedrin inclusion bodies - Aqueous Process

Ammonium hydroxide solution (28% NH₃) is added to a mixture of Eudragit®S100 (62 g, Röhm Pharma Co.) and PEG 400 (6.2 g, poly(ethylene glycol) average M.W. 400, Aldrich Chemical Co.) in deionized water (551.8 g) until a pH of about 9.4 is obtained. The resulting mixture is stirred for 30 minutes to obtain a solution. V8vEGTDEL polyhedrin inclusion bodies (62 g, average size about 2 µm, about 1011 bodies per gram), Blancophor BBH (248 g, stilbene brightner, Miles Inc.) and CYASORB®UV 9 (31 g, average particle size about 2 µm, Cytec Ind.) are added to the pH adjusted aqueous solution. The resultant mixture is stirred for 30 minutes and air dried with blending to obtain solid granules. The solid granules are air milled to give coated V8vEGTDEL polyhedrin inclusion bodies (362 g, average size about 5 µm). The coated V8vEGTDEL polyhedrin inclusion bodies prepared above are identified as composition 1 in Table II.

Using essentially the same procedure, but using the ingredients listed in Table I, the coated pesticidal agents identified as compositions 2-10 in Table II are prepared.

TABLE I

Pesticidal Agent

- a. V8vEGTDEL polyhedrin inclusion bodies
- b. *Heliothis zea* NPV
- c. AcMNPV
- d. 1:1 mixture of *Heliothis zea* NPV and AcMNPV

pH-Dependent Polymer

- e. Eudragit®S100

Plasticizer

- f. PEG 400

UV-Protector

- g. CYASORB®UV 9
- h. Charcoal

Stilbene Compound

- i. Blancophor BBH

Glidant

- j. Talc

TABLE II

Coated Pesticidal Agent Compositions prepared by the Aqueous Process

Composition Number	Ingredient / wt/wt%				
	Pesticidal Agent	pH-Dependent Polymer	Plasticizer	UV- Protector	Stilbene Compound
1	a/15.15	e/15.15	f/1.52	g/7.58	i/60.61
2	a/24.69	e/12.35	f/1.23	g/12.35	i/49.38
3	a/21.74	e/21.74	f/2.17	g/10.87	i/43.48
4	a/19.80	e/9.90	f/0.99	g/9.90	i/49.41
5	a/17.86	e/17.86	f/1.79	g/8.93	i/53.57
6	a/16.53	e/8.26	f/0.83	g/8.26	i/66.12
7	b/4.81	e/11.75	f/1.18	g/11.75	i/70.51
8	c/21.36	e/9.71	f/0.97	g/9.71	i/58.25
9	d/8.41	e/11.98	f/1.20	g/11.43	i/66.98
10	a/16.09	e/3.17	f/0.32	h/32.17	i/32.17
					j/8.04

EXAMPLE 2**Preparation of coated V8vEGTDEL polyhedrin inclusion bodies - Organic Process**

V8vEGTDEL polyhedrin inclusion bodies (43.24 g, average size about 2 μm , about 10^{11} bodies per gram), Blancophor BBH (86.44g) and CYASORB®UV 9 (31.93 g) are added to a solution of Eudragit®S100 (5.58 g) and PEG 400 (1.51 g) in a 30:70 acetone/isopropanol solution (195.15 g). The resultant mixture is stirred for several minutes and air dried with blending to obtain solid granules. The solid granules are milled through a 60 mesh screen to give coated V8vEGTDEL polyhedrin inclusion bodies having an average size of about 10 μm . The coated V8vEGTDEL polyhedrin inclusion bodies prepared above are identified as composition 11 in Table IV.

Using essentially the same procedure, but using the ingredients listed in Table III, the coated pesticidal agents identified as compositions 12-28 in Table IV are prepared.

TABLE III**Pesticidal Agent**

- a. V8vEGTDEL polyhedrin inclusion bodies
- b. Wild gypsy moth NPV

pH-Dependent Polymer

- c. Eudragit®S100
- d. Cypress®48 (maleic anhydride/styrene copolymer, Cytec Ind.)

Plasticizer

- e. PEG 400

UV-Protector

- f. CYASORB®UV 9
- g. Charcoal
- h. TiO_2

Stilbene Compound

- i. Blancophor BBH

Disintegrating Agent

- j. MORWET®D425

Stabilizer

- k. Trehalose

TABLE IV

Coated Pesticidal Agent Compositions prepared by the Organic Process

Composition Number	Ingredient / wt/wt%						
	Insecticidal Pathogen	pH-Dependent Polymer	Plasticizer	UV- Protector	Stilbene Compound	Disinte- grating Agent	Stabilizer
11	a/23.76	c/3.24	e/0.83	f/17.55	i/47.50	j/1.12	-
12	a/70.18	c/3.51	e/1.75	f/17.54	-	j/7.02	-
13	a/35.09	c/3.86	e/1.75	f/17.54	i/34.74	j/7.02	-
14	a/23.40	c/4.91	e/1.75	f/17.52	i/45.39	j/7.02	-
15	b/77.19	c/3.51	e/1.75	f/17.54	-	-	-
16	b/62.86	c/5.71	e/2.86	f/28.57	-	-	-
17	b/40.00	c/3.64	e/1.82	f/18.18	-	-	k/36.36
18	b/77.19	c/3.51	e/1.75	g/17.54	-	-	-
19	b/62.86	c/5.71	e/2.86	g/28.57	-	-	-
20	b/77.19	c/3.51	e/1.75	h/17.54	-	-	-

Coated Pesticidal Agent Compositions prepared by the Organic Process

Composition Number	Insecticidal Pathogen	pH-Dependent Polymer	Ingredient / wt/wt%				Disinte- grating Agent	Stabiliz r
			Plasticizer	UV- Protector	Stilbene Compound			
21	b/62.86	c/5.71	e/2.86	h/28.57	-	-	-	
22	b/26.83	c/4.05	e/2.02	g/13.42	-	-	k/53.67	
23	b/44.74	c/2.24	e/1.12	f/11.22	i/40.67	-	-	
24	b/23.16	c/2.11	e/1.05	f/10.53	i/63.16	-	-	
25	b/40.00	c/3.64	e/1.82	g/18.18	i/36.36	-	-	
26	b/20.37	c/3.70	e/1.85	h/18.52	i/55.56	-	-	
27	b/48.89	c/4.44	e/2.22	f/22.22	-	j/22.22	-	
28	b/23.66	c/3.57	e/1.79	g/11.83	-	j/11.83	k/47.33	

EXAMPLE 3**Preparation of wettable powder pesticidal compositions**

5 The coated V8vEGTDEL polyhedrin inclusion bodies identified as composition 1 in Table II (362 g) are added to a premix of MORWET®EFW (13.1 g), MORWET®D425 (26.2 g), kaolin clay (91.6 g), synthetic calcium sulfate (6.5 g, MICRO-CEL®E, Manville Co.) and citric acid (0.7 g). The resultant mixture is blended to obtain the wettable powder composition identified as composition 29 in Table V.

10 Using essentially the same procedure, the wettable powder compositions identified as compositions 30-53 in Table V are prepared.

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TABLE V
Wettable Powder Pesticidal Compositions

Composition Number	Coated Pesticidal Agent ¹	Ingredient / wt/wt%					Citric Acid	Sugar	MIRA-SPERSE ²
		MORWET [®] EFW	MORWET [®] D425	Kaolin Clay	MICRO-CEL [®] E				
29	1/72.40	2.62	5.24	18.32	1.30		0.14	-	-
30	2/38.83	5.80	11.60	40.58	2.90		0.29	-	-
31	3/37.50	5.92	11.85	41.47	2.96		0.30	-	-
32	4/41.67	5.53	11.06	38.70	2.76		0.28	-	-
33	5/33.33	6.32	12.64	44.23	3.16		0.32	-	-
34	6/35.09	6.15	12.31	43.07	3.08		0.31	-	-
35	7/38.25	5.85	11.71	40.97	2.93		0.29	-	-
36	8/43.25	5.38	10.76	37.65	2.69		0.27	-	-
37	9/39.50	5.73	11.47	40.14	2.87		0.29	-	-
38	11/29.08	6.76	13.51	47.27	3.38		-	-	-
39	15/13.36	13.21	4.40	29.39	4.40		-	17.62	17.62
40	16/13.78	13.15	4.38	29.24	4.38		-	17.53	17.53

¹ The coated pesticidal agent is identified by the composition number from Table II or Table IV.

² MIRA-SPERSE[®] is a 2-hydroxypropyl ether starch available from A. E. Staley Manufacturing Co., Decatur IL.

TABLE V (Continued)
Wettable Powder Pesticidal Compositions

Composition Number	Coated Pesticidal Agent ¹	Ingredient / wt/wt%					
		MORWET [®] EFW	MORWET [®] D425	Kaolin Clay	MICRO-CEL [®] E	Citric Acid	Sugar MIRA-SPERSE [®] 2
41	17/23.62	11.65	3.88	25.90	3.88	-	15.52
42	18/13.01	13.27	4.42	29.50	4.42	-	17.68
43	19/15.97	12.81	4.27	28.50	4.27	-	17.08
44	20/13.52	13.19	4.39	29.34	4.39	-	17.58
45	21/16.70	12.69	4.23	28.24	4.23	-	16.94
46	22/22.61	11.80	3.93	26.21	3.93	-	15.78
47	23/21.95	11.90	3.96	26.47	3.96	-	15.87
48	24/43.78	8.57	2.86	19.07	2.86	-	11.42
49	25/29.99	10.68	3.56	23.79	3.56	-	14.23
50	26/48.56	7.84	2.61	17.45	2.61	-	10.46
51	27/27.64	11.03	3.67	24.57	3.67	-	14.74
52	28/18.85	12.37	4.12	27.53	4.12	-	16.49
53	10/30.45	6.59	13.19	46.16	3.30	0.33	-

EXAMPLE 4**Insecticidal evaluations of non-irradiated and irradiated wettable powder pesticidal compositions against *L. dispar***

Wettable powder insecticidal pathogen compositions are suspended in distilled water and diluted to a concentration of 2.4×10^5 coated insecticidal polyhedrin inclusion bodies per mL. The resultant suspension (0.5 mL) is pipetted to the surface of wheat germ diet in a 180 mL plastic cup. Each cup is exposed to ultraviolet radiation (one Westinghouse BLB bulb and one Phillips F40 UVB bulb set three inches apart, the distance from the center line of the radiation source and the diet surface is 4 inches) for either 0 or 80 minutes. Ten second instar *L. dispar* caterpillars are placed in each cup. The cups are covered and maintained in darkness at 29°C, 55-60% relative humidity. After 13 days, the cups are examined and mortality by virus infection is determined.

The results are summarized in Table VI wherein the effectiveness of each composition is expressed as the percent of original activity remaining after ultraviolet exposure (% OAR), i.e. % mortality caused by irradiated composition + % mortality caused by non-irradiated composition x 100. The control composition used in the evaluations is identified below.

Control Composition

<u>Ingredient</u>	<u>wt/wt%</u>
¹ Coated insecticidal pathogen	11.19
MORWET®EFW	13.54
MORWET®D425	4.51
Kaolin Clay	30.12
MICRO-CEL®E	4.51
Sugar	18.06
MIRA-SPERSE®	18.06

¹ 93.62 wt/wt% wild gypsy moth NPV, 4.26% wt/wt% Eudragit® S100, and 2.13 wt/wt% PEG 400.

TABLE VI**Insecticidal Evaluations Against *L. dispar***

Composition Number ¹	% Mortality		
	<u>Irradiated</u>	<u>Non- Irradiated</u>	<u>% OAR²</u>
Control	47.5	100.0	47.5
39	70.0	100.0	70.0
40	76.7	99.2	77.2
41	87.5	99.2	88.2
42	91.7	98.3	93.2
43	98.3	99.2	99.2
44	57.5	100.0	57.5
45	65.0	96.7	67.3
46	82.5	98.3	84.0
47	91.7	98.3	93.2
48	90.0	99.2	90.8
49	90.0	98.3	91.6
50	87.9	100.0	87.9
51	77.5	99.2	78.2
52	90.0	99.2	90.7

¹ Composition number from Table V.² Percentage of original activity remaining after ultraviolet exposure.**EXAMPLE 5****Insecticidal evaluations of wettable powder pesticidal compositions against *Helicoverpa zea***

Plastic bioassay trays containing 32 open-faced wells (4 x 4 x 2.5 cm, L x W x H, C-D International, Inc.) per tray are utilized as test arenas in this evaluation. Five mL of Stoneville diet (soybean/wheat germ) is poured into each tray-well and allowed to harden. Aqueous suspensions (0.4 mL) of wettable powder insecticidal pathogen compositions are evenly spread over the surface of the hardened diet to provide from 4×10^5 to 4×10^7 V8vEGTDEL coated or uncoated polyhedrin inclusion bodies per well. After drying the trays in a laminar flow hood, one three-day-old *Helicoverpa zea* larvae is placed on the surface of the diet in each tray-well. The wells are covered with an adhesive, vented clear plastic sheet (C-D International, Inc.), held under constant fluorescent light and at a temperature of about 27°C. Five days and ten days after treatment, the wells are examined and larval mortality measurements are made.

The results are summarized in Table VII. The control compositions used in the evaluations are identified below.

Control Composition	Ingredient	wt/wt%
A	Uncoated V8vEGTDEL polyhedrin inclusion bodies	10.00
	MORWET®EFW	8.53
	MORWET®D425	17.06
	Kaolin Clay	59.71
	MICRO-CEL®E	4.27

Continuation of the Table on the next page

(continued)

Control Composition	Ingredient	wt/wt%
B	Citric Acid	0.43
	Uncoated V8vEGTDEL polyhedrin inclusion bodies	8.69
	MORWET®EFW	8.71
	MORWET®D425	17.40
	Kaolin Clay	60.86
	MICRO-CEL®E	4.35

TABLE VIIInsecticidal Evaluations Against *Helicoverpa zea*

Composition Number ¹	Concentration (bodies/well)	% Mortality	
		5 Days	10 Days
Control A	4 x 10 ⁷	49	91
	4 x 10 ⁶	47	93
	4 x 10 ⁵	29	63
Control B	4 x 10 ⁷	33	75
	4 x 10 ⁶	35	70
	4 x 10 ⁵	18	47
29	4 x 10 ⁷	77	100
	4 x 10 ⁶	58	100
	4 x 10 ⁵	35	77
38	4 x 10 ⁷	43	95
	4 x 10 ⁶	45	80
	4 x 10 ⁵	25	56

¹ Composition number from Table V.**EXAMPLE 6****Evaluation of non-irradiated and irradiated wettable powder compositions against *H. zea* and *H. virescens***

Plastic bioassay trays containing 32 open-faced wells (4 x 4 x 2.5 cm, L x W x H, C-D International, Inc.) per tray are utilized as test arenas in this evaluation. Five mL of Stoneville diet (soybean/wheat germ) is poured into each tray-well and allowed to harden. Aqueous suspensions (0.4 mL) of the wettable powder insecticidal pathogen compositions are evenly spread over the surface of the hardened diet to provide 4 x 10⁶ V8vEGTDEL coated or uncoated polyhedrin inclusion bodies per well. Some of the treated trays are then held under ultraviolet lamps (two FS40UVB bulbs set 30

cm above the trays, Phillips Co.) for either one or two hours. Trays selected for two hours of irradiation are provided with an additional 0.4 mL of deionized water per well at the one hour time interval to prevent the diet from over-drying and cracking. All trays are then infested with a single three-day-old *H. zea* or four-day-old *H. virescens* larvae. The wells are covered with an adhesive, vented clear plastic sheet (C-D International, Inc.), held under constant fluorescent light and at a temperature of about 27°C. Ten days after treatment, the wells are examined and larval mortality measurements are made.

The results are summarized in Table VIII. The control composition used in the evaluations is identified below.

Control Composition	
Ingredient	wt/wt%
Uncoated V5vEGTDEL polyhedrin inclusion bodies	4.93
MORWET®EFW	9.01
MORWET®D425	18.02
Kaolin Clay	63.09
MICRO-CEL®E	4.51
Citric Acid	0.45

TABLE VIII

Evaluation of non-irradiated and irradiated wettable powder compositions against *H. zea* and *H. virescens*

Composition Number	Irradiation Exposure (hours)	Mean % Larval Mortality	
		<i>H. zea</i>	<i>H. virescens</i>
Control	0	59	77
	1	46	56
	2	40	36
53 ¹	0	91	89
	1	90	87
	2	83	56

¹ Composition number from Table V.

EXAMPLE 7

Solvent compatibility evaluations

The following evaluation is used to determine the effect of various solvents and mixtures thereof on the activity of *Autographa californica* polyhedrin inclusion bodies. A mixture of *Autographa californica* polyhedrin inclusion bodies (0.55 g) and the appropriate solvent or solvent mixture (1.5 mL) is held in a conical tube for 10 or 60 minutes. The tubes are then centrifuged and the supernatant is decanted. The solids are dried under vacuum in a dessicator. The dried solids are then evaluated against *Heliothis virescens* according to the procedure described in Example 6 (no irradiation).

The results are summarized in Table IX. As can be seen from the data in Table IX, the *Autographa californica* polyhedrin inclusion bodies mixed with methylene chloride for 10 and 60 minutes are significantly less active against

Heliothis virescens than the bodies mixed with acetone, isopropanol and a 30:70 acetone/isopropanol mixture.

TABLE IX

Solvent Compatibility Evaluations			
Solvent	Time held (minutes)	Rate (bodies/well)	% Mortality
Acetone	10	4×10^2	19
	60	4×10^2	25
	10	4×10^4	100
	60	4×10^4	100
Isopropanol	10	4×10^2	31
	60	4×10^2	13
	10	4×10^4	100
	60	4×10^4	100
Acetone/Isopropanol (30:70)	10	4×10^2	34
	60	4×10^2	19
	10	4×10^4	100
	60	4×10^4	100
Methylene chloride	10	4×10^2	9
	60	4×10^2	6
	10	4×10^4	84
	60	4×10^4	19

Claims

1. A process for the preparation of a coated pesticidal agent which comprises:

a) preparing an aqueous mixture of a pH-dependent polymer and optionally a plasticizer;

b) dissolving the pH-dependent polymer by adjusting the pH of the mixture of step (a) to a pH above the solubilization pH of the pH dependent polymer;

c) adding a pesticidal agent, an ultraviolet protector, optionally a stilbene compound, optionally a disintegrating agent and optionally a glidant to the solution of step (b), and blending to produce a homogeneous suspension;

d) drying the homogeneous suspension of step (c); and optionally

e) milling the dried material of step (d).

2. The process according to claim 1 wherein the pH-dependent polymer is selected from the group consisting of a methacrylic acid and methyl methacrylate copolymer, a mixture of methacrylic acid and methyl methacrylate copolymers and a maleic anhydride and styrene copolymer; the plasticizer is selected from the group consisting of a polyethylene glycol, a polypropylene glycol, diethyl phthalate, dibutyl phthalate, a citric acid ester, castor oil and triacetin; the base is selected from the group consisting of ammonium hydroxide, an alkali metal hydroxide and an alkaline earth metal hydroxide; the pesticidal agent is an insecticidal pathogen; the ultraviolet protector is selected from the group consisting of carbon black, a benzophenone, a dye and titanium dioxide; the pH is adjusted in step (b) to pH 8.5 to 10; the disintegrating agent is selected from the group consisting of salts of the condensation products of formaldehyde with the sulfonation products of polycyclic aromatic compounds, a hydrophilic starch, carboxy methylcellulose and polyvinyl pyrrolidone; and the glidant is selected from the group consisting of talc, magnesium stearate, calcium stearate and calcium sulfate.

3. The process according to claim 2 wherein the plasticizer is a polyethylene glycol having a molecular weight of about

300 to 1,000; the base is ammonium hydroxide; the insecticidal pathogen is a virus selected from the group consisting of wild gypsy moth NPV, AcMNPV E2, AcMNPV L1, AcMNPV V6, V8vEGTDEL, V8vEGTDEL-AaIT and *Heliothis zea* NPV; the disintegrating agent is a sodium sulfonate of a naphthalene formaldehyde condensate; and the glidant is talc and wherein the coated pesticidal agent has a particle size less than about 20 μ m.

4. A process for the preparation of a coated pesticidal agent which comprises:

a) blending a mixture of a pH-dependent polymer, a pesticidal agent, optionally a plasticizer, an ultraviolet protector, optionally a stilbene compound, optionally a disintegrating agent and optionally a glidant in a organic solvent selected from the group consisting of acetone, a C₁-C₃alcohol, and mixtures thereof;

b) drying the homogeneous suspension of step (a); and optionally

c) milling the dried material of step (b).

5. The process according to claim 4 wherein the organic solvent is a mixture of acetone and a C₁-C₃alcohol and the ratio of acetone to the C₁-C₃alcohol is about 1:9 to 9:1.

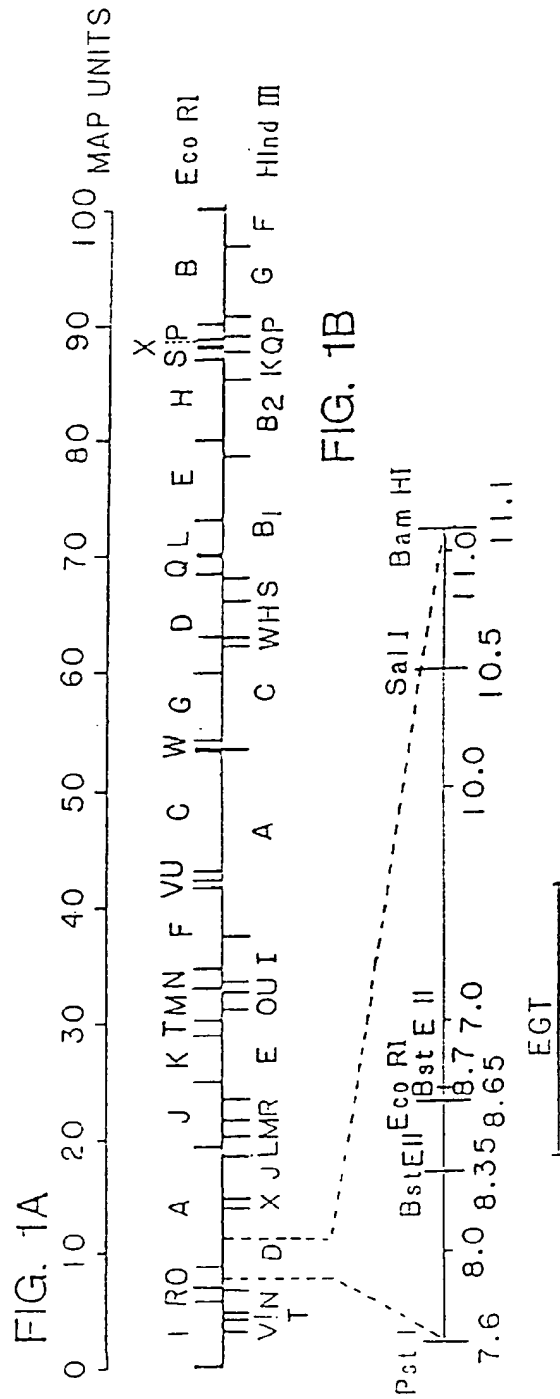
6. The process according to claim 4 wherein the pH-dependent polymer is selected from the group consisting of a methacrylic acid and methyl methacrylate copolymer, a mixture of methacrylic acid and methyl methacrylate copolymers and a maleic anhydride and styrene copolymer; the pesticidal agent is an insecticidal pathogen; the plasticizer is selected from the group consisting of a polyethylene glycol, a polypropylene glycol, diethyl phthalate, dibutyl phthalate, a citric acid ester, castor oil and triacetin; the ultraviolet protector is selected from the group consisting of carbon black, a benzophenone, a dye and titanium dioxide; the disintegrating agent is selected from the group consisting of salts of the condensation products of formaldehyde with the sulfonation products of polycyclic aromatic compounds, a hydrophilic starch, carboxymethyl cellulose and polyvinyl pyrrolidone; the glidant is selected from the group consisting of talc, magnesium stearate, calcium stearate and calcium sulfate; and the C₁-C₃alcohol is isopropanol.

7. A coated pesticidal agent which comprises a pesticidal agent core surrounded by a matrix which comprises about 2 to 25% by weight of a pH-dependent polymer, 0% to about 5% by weight of a plasticizer, about 5 to 45% by weight of an ultraviolet protector, 0% to about 75% by weight of a stilbene compound, 0% to about 10% by weight of a disintegrating agent, and 0% to about 10% by weight of a glidant.

8. The coated pesticidal agent according to claim 7 wherein the ratio of the pesticidal agent to the matrix is about 1:1 to 1:10.

9. The coated pesticidal agent according to claim 7 wherein the pesticidal agent is an insecticidal pathogen; the pH-dependent polymer is selected from the group consisting of a methacrylic acid and methyl methacrylate copolymer, a mixture of methacrylic acid and methyl methacrylate copolymers and a maleic anhydride and styrene copolymer; the plasticizer is selected from the group consisting of a polyethylene glycol, a polypropylene glycol, diethyl phthalate, dibutyl phthalate, a citric acid ester, castor oil and triacetin; the ultraviolet protector is selected from the group consisting of carbon black, a benzophenone, a dye and titanium dioxide; the disintegrating agent is selected from the group consisting of salts of the condensation products of formaldehyde with the sulfonation products of polycyclic aromatic compounds, a hydrophilic starch, carboxy methyl-cellulose and polyvinyl pyrrolidone; and the glidant is selected from the group consisting of talc, magnesium stearate, calcium stearate and calcium sulfate.

10. A wettable powder pesticidal composition which comprises about 2 to 25% by weight of a wetting agent; about 2 to 40% by weight of a dispersing agent; about 10 to 70% by weight of a bulking agent; about 1 to 10% by weight of a flow enhancing agent; 0% to about 20% by weight of a pH-modifying agent; and about 5 to 75% by weight of a coated pesticidal agent which comprises a pesticidal agent core surrounded by a matrix which comprises about 2 to 25% by weight of a pH-dependent polymer, 0% to about 5% by weight of a plasticizer, about 5 to 45% by weight of an ultraviolet protector, 0% to about 75% by weight of a stilbene compound, 0% to about 10% by weight of a disintegrating agent, and 0% to about 10% by weight of a glidant.



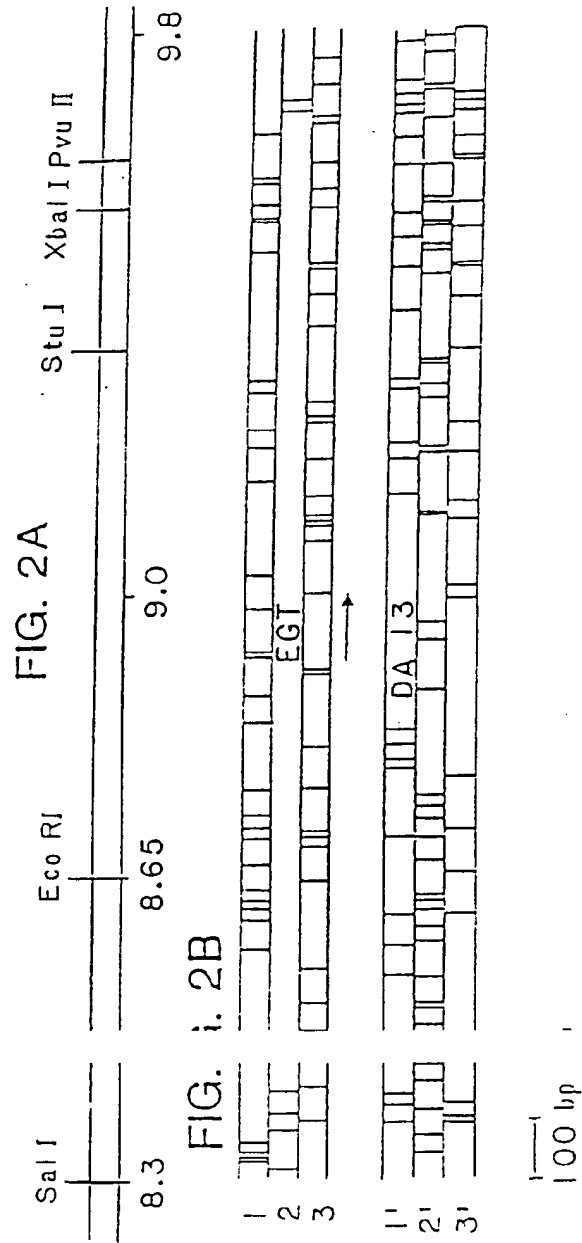


Figure 3A

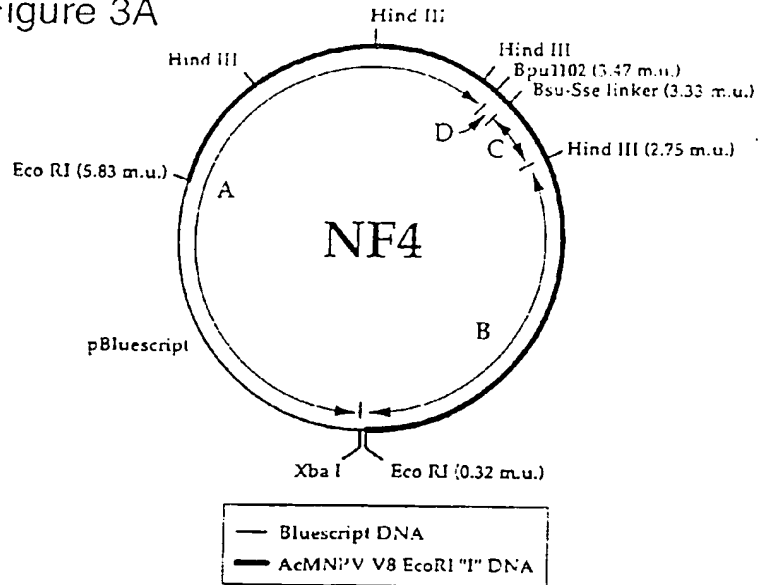


Figure 3B

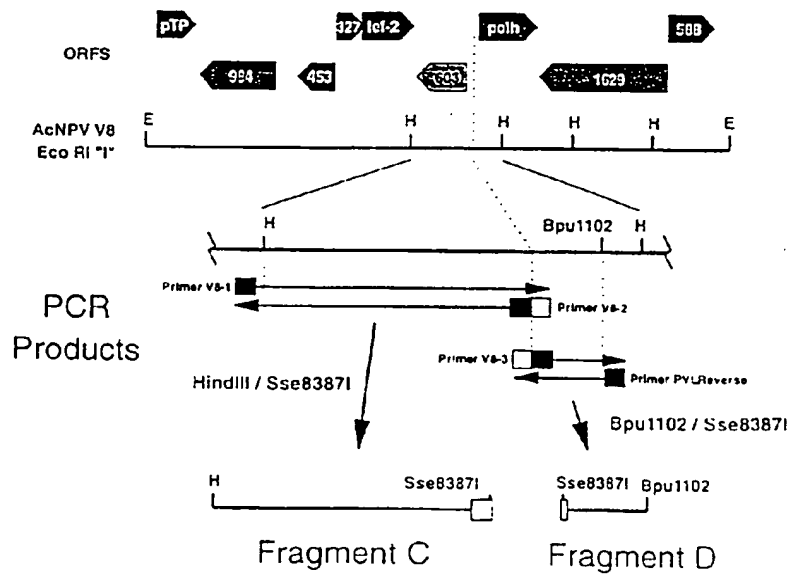
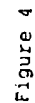


Figure 3



Esp 3I-based Modular Expression Vectors

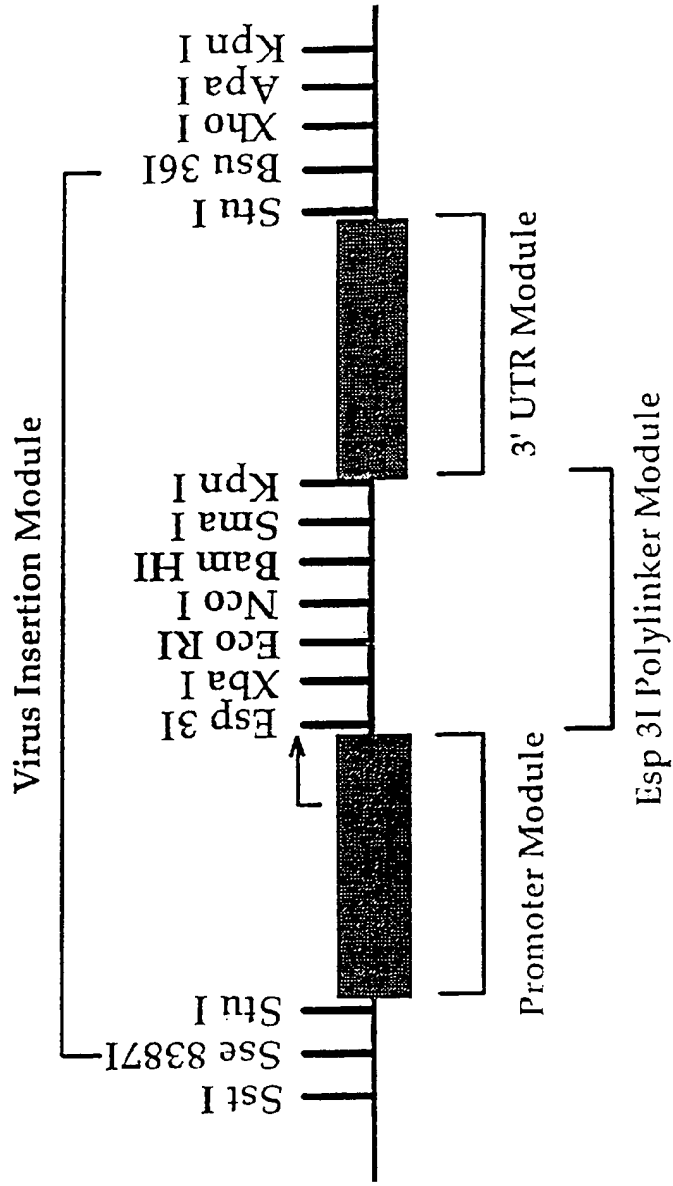


Figure 6

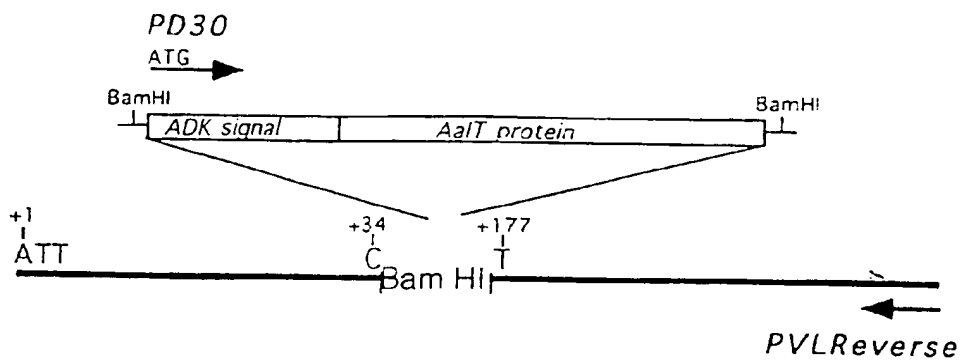


Figure 6

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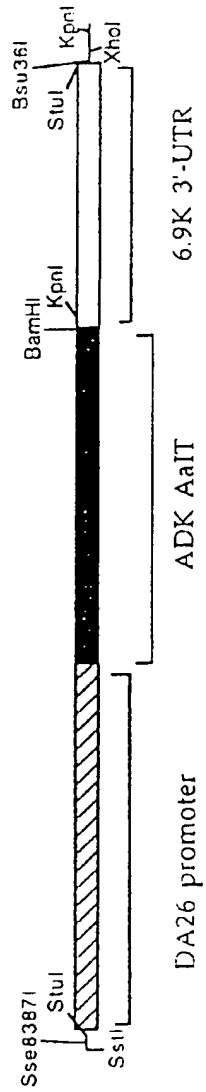


Figure 7



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Application Number
EP 95 30 5237

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X A	WO-A-92 19102 (LIM LABORATORIES INC.) * page 16, line 1 - page 17, line 19; claims; examples 1,3 * ---	7 1-6,8-10	A01N25/26 A01N63/00 A01N63/04 //(A01N63/00, 25:26), (A01N63/04, 25:26)
X A D	WO-A-89 04170 (LIM TECHNOLOGY LABORATORIES INC.) * page 14, line 1 - line 19; claims; example 11 * * page 11, line 16 - line 28 * & US-A-4 948 586 (H.A. BOHM ET AL) ---	4 6-10	
X A A	WO-A-89 07447 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) * page 7, line 1 - page 8, line 22; claims * --- US-A-5 124 149 (M. SHAPIRO ET AL) * column 2, line 24 - line 44 * --- EP-A-0 250 908 (DOW CHEMICAL COMPANY) * page 5, line 8 - line 24; examples 1,5,7 * ---	4 7 1,2,7,9	
A	DATABASE WPI Section Ch, Week 8651, Derwent Publications Ltd., London, GB; Class A12, AN 86-335063/51 & JP-A-61 249 904 (NIPPON KAYAKU KK) 7 November 1986 * abstract * --- -/--	10	TECHNICAL FIELDS SEARCHED (Int.Cl.6) A01N
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 27 November 1995	Examiner Fletcher, A
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		I : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons - - - - - & : member of the same patent family, corresponding document	

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EUROPEAN SEARCH REPORT

Application Number
EP 95 30 5237

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
A	JOURNAL OF ECONOMIC ENTOMOLOGY, vol.83, no.1, February 1990, COLLEGE PARK, MARYLAND US pages 168 - 172 M.SHAPIRO ET AL 'Laboratory Evaluation of Dyes as Ultraviolet Screens for the Gypsy Moth (Lepidoptera: Lymantriidae) Nuclear Polyhedrosis Virus' -----		
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 27 November 1995	Examiner Fletcher, A
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>I : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons</p> <p>..... A : member of the same patent family, corresponding document</p>			

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